

Production of CO₂ in Crude Oil Bioremediation in Clay Soil

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ABSTRACT

The aim of the present work was to evaluate the biodegradation of petroleum hydrocarbons in clay soil a 45-days experiment. The experiment was conducted using an aerobic fixed bed reactor, containing 300g of contaminated soil at room temperature with an air rate of 6 L/h. The growth medium was supplemented with 2.5% (w/w) (NH₄)₂SO₄ and 0.035% (w/w) KH₂PO₄. Biodegradation of the crude oil in the contaminated clay soil was monitored by measuring CO₂ production and removal of organic matter (OM), oil and grease (OandG), and total petroleum hydrocarbons (TPH), measured before and after the 45-days experiment, together with total heterotrophic and hydrocarbon-degrading bacterial count. The best removals of OM (50%), OandG (37%) and TPH (45%) were obtained in the bioreactors in which the highest CO₂ production was achieved.

Key words: Biostimulation; crude oil; clay soil

INTRODUCTION

Brazil has intensified efforts on biotechnology research, especially on soil bioremediation, aiming to solve some environmental impacts resulting from accidental oil spills. One of the greatest advantages on the study of bioremediation in crude oil contaminated soil treatment is its cost-effectiveness, as compared to some physico-chemical techniques, which are expensive and need continuous monitoring in order to attain successful results. Bioremediation has emerged as a good technique for environmental treatment regarding organic compounds, such as petroleum hydrocarbons, due to its flexibility and adaptability in different sites (Ryan, 1991).

Despite been greatly adjustable, there are some factors, which influence the bioremediation process and should be monitored. These include temperature, type of soil, pollutants type and concentration, nutrients and oxygen availabilities

and microorganisms' concentration on the impacted site. Therefore, there is a need to adjust some environmental conditions such as improving soil aeration, monitoring and correcting the moisture and pH in order to stimulate the indigenous microorganism activity and to obtain the best pollutant removals.

Treating soil with high clay content is a great challenge since that kind of particle has a deleterious effect on mass transfer, blocking air and water from passing, thus affecting the aerobic process. Moreover, the organic pollutants attach to the soil matrix, reducing their bioavailability to microorganisms. In some cases, treatment of contaminated soils needs biostimulation of indigenous microorganisms (Cunningham and Philp, 2000). Nitrogen and phosphorus are the usual nutrients, which have been used in biostimulation process in order to support microbial growth (Liebeg and Cutright, 1999). These nutrients have been considered as limitant

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factors for the bioremediation of contaminated soils, but when used properly, they may stimulate soil biodegradation (Zhou and Crawford, 1995).

The main objective of the present work was to evaluate biodegradation of petroleum hydrocarbons in clay soil, supplemented with nitrogen and phosphorus in order to stimulate native microorganisms.

MATERIALS AND METHODS

Contaminated Soil

The contaminated soil was collected in 2000 from the state of Sergipe/Brazil. Samples were taken from three distinct spots of the contaminated area, in order to obtain a representative material of the soil. The material was stored at 4°C. To perform

the experimental work, the samples were thoroughly homogenized.

Experimental Apparatus

The biodegradability experiments were held in four aerobic fixed bed reactors (**D1**, **D2**, **D3** and **D4**), with 300g of contaminated soil at room temperature and aeration rate of 6 L/h, during 45 days. The bioreactors were completely closed in order to avoid CO₂ leakage to the environment before passing through each CO₂ removal tubes. In each of them a solution containing Ba(OH)₂, meant to collect CO₂ evolved from biodegradation, was added. Fig. 1 shows the set of the experimental apparatus for CO₂ dosage, consisting of a bottle of NaOH solution (60%), in order to remove CO₂ from the incoming air, a bioreactor and tube series with Ba(OH)₂.

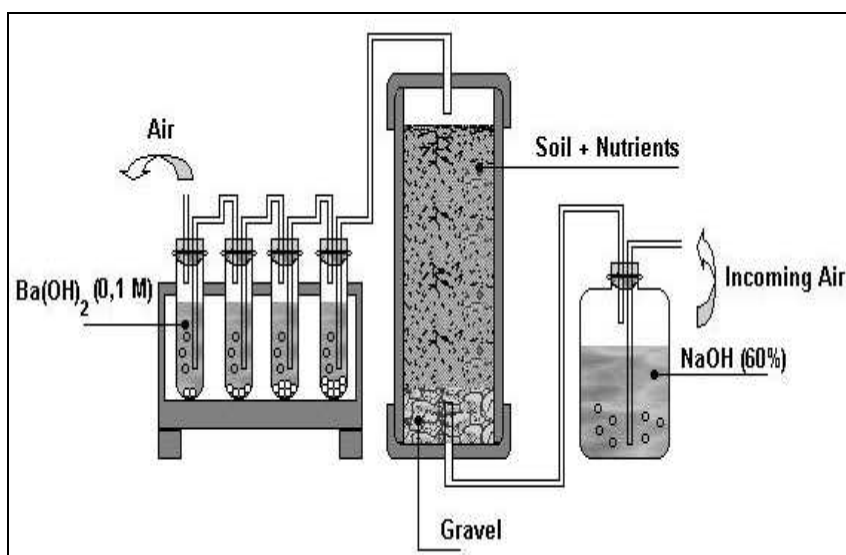


Figure 1 - Scheme of an Aerobic Fixed Bed Reactor for Collecting CO₂ Evolved from Biodegradation.

The medium was supplemented with (NH₄)₂SO₄ (2.5% w/w) and KH₂PO₄ (0.035% w/w). In order to verify crude oil biodegradation, organic matter (OM), oil and grease (OandG) and total petroleum hydrocarbon (TPH) content was determined before and after 45 days period. In addition, total heterotrophic (BHT) and hydrocarbon-degrading bacterial (BHc) count was also determined before and after the 45-days test.

Physico-chemical Analysis and Bacterial Count

The organic matter content (OM) was measured by using Walkey and Black Method (Jaramillo, 1996), while for oil and grease content (OandG), an adaptation of the methodology described in section 5520 D of the Standard Methods (APHA, 1992) was applied. Total petroleum hydrocarbons (TPH) were extracted with solvent S-316 and measured in infrared equipment OCMA-350 (Horiba).

Nitrogen content in the contaminated soil was measured utilizing Kjeldahl method and phosphorous by using the total phosphorous method (Jaramillo, 1996). Soil pH was determined by Jaramillo's method (1996). In order to determine the soil's water content, a thermogravimetric balance (IV 2000 GEHAKA) was used. For water-holding capacity of soil, the measurement was performed using the method described by Santos (2001).

CO₂ content was measured by a modified version of the method from Gledhill's Test in a closed system. The analysis was adapted for each type of tested soil (IBAMA, 1988). Total heterotrophic bacteria were counted according to spread-plated method on Tryptic Soy Agar (TSA) medium and the results were expressed in colony-forming units (CFU) per gram of dry weight. Hydrocarbon-degrading bacteria (BHc) count was done according to Most Probable Number method (MPN) (Volpon et al., 1997) and the unit was expressed in MPN/g of dry weight.

RESULTS AND DISCUSSION

Soil Characterization

The main physico-chemical and microbiological characteristics of the contaminated soil were evaluated and shown in Table 1.

High levels of carbon content were due to the presence of crude oil in the soil, which in fact was related to oil and grease (OandG) and total petroleum hydrocarbon (TPH). According to The Dutch Target and Intervention Values, a soil needs intervention when TPH contents exceed 5g/Kg, in a soil containing 25% clay and 10% organic matter content (Johnson, 2003a). According to the Danish (Zealand) Guidelines, the soil can be regarded as contaminated when TPH content exceeds 0.2g/Kg (Johnson, 2003b). Therefore, the investigated soil showed high levels of TPH by all the International Standards evaluated.

Table 1 - Physico-chemical and Microbiological Characterization of the Soil

Parameter	Value
Carbon (g/Kg)	44.8±0.2
Nitrogen (g/Kg)	2.9±0.2
Phosphorus (mg/Kg)	6±1
Organic Matter (g/Kg)	77.2±0.4
Oil and Grease (g/Kg)	27.5±1.5
TPH (g/Kg)	9.7±0.1
pH	7.5±0.1
Moisture Content (%)	53.6±0.3
Water-holding capacity (%)	73.6±0.3
Density of Particles (g/cm ³)	1.8±0.0
Total Heterotrophic Bacteria (CFU/g)	9.2x10 ⁶
Hydrocarbon-degrading Bacteria (MPN/g)	2.2x10 ³

The soil in question had a neutral pH, being thus suitable for contaminant biodegradation by indigenous microorganisms. However, the moisture content was high and could influence negatively biodegradability, as a good aeration of the soil would be difficult to attain. In order to amend the moisture content, the soil was dried at

room temperature until it reached its 50% water-holding capacity.

According to Jaramillo's method (1996), the tested soil was rich in nitrogen and poor in phosphorus, so that it had to be supplemented on nutritional shortage, by adding (NH₄)₂SO₄ and KH₂PO₄, it was done according to the results from experimental design (Baptista et al., 2003).

As far as soil microbial population is concerned, representative values for crude oil biodegradation could be verified when compared to the results from Jorgensen et al. (2000). Total heterotrophic and hydrocarbon-degrading bacteria 8.2×10^7 CFU/g and 5.4×10^4 MPN/g, respectively, which could degrade n-alkanes from 50 to 90% of n-alkanes after 5 months.

Evaluation of CO₂ Production in Biodegradation Assays

Monitoring of CO₂ produced during 45 days-assays in the aerobic fixed bed reactors are shown

in Fig. 2. **B1** and **B2** were control bioreactors in which there was no nutrients addition, while reactors **D1**, **D2**, **D3** and **D4** were supplemented due to nutritional shortage.

As demonstrated in Fig. 2, bioreactor **D2** showed the best CO₂ production per soil's mass (4,000 mg CO₂/Kg). The production of gas was approximately 3,000 mg CO₂/Kg in other bioreactors. Bioreactor **D3** did not show significant changes CO₂ production after the twenty-ninth day of assay, while bioreactor **D4** maintained constant production only after fortieth day.

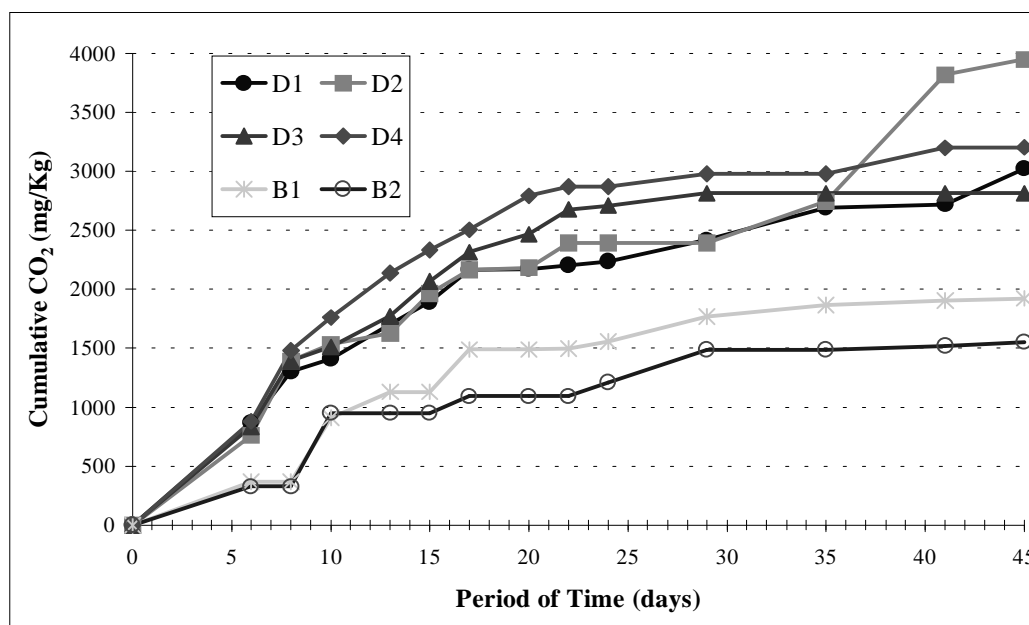


Figure 2 - Produced CO₂ Monitoring During Biodegradability Assays in Aerobic Bioreactors.

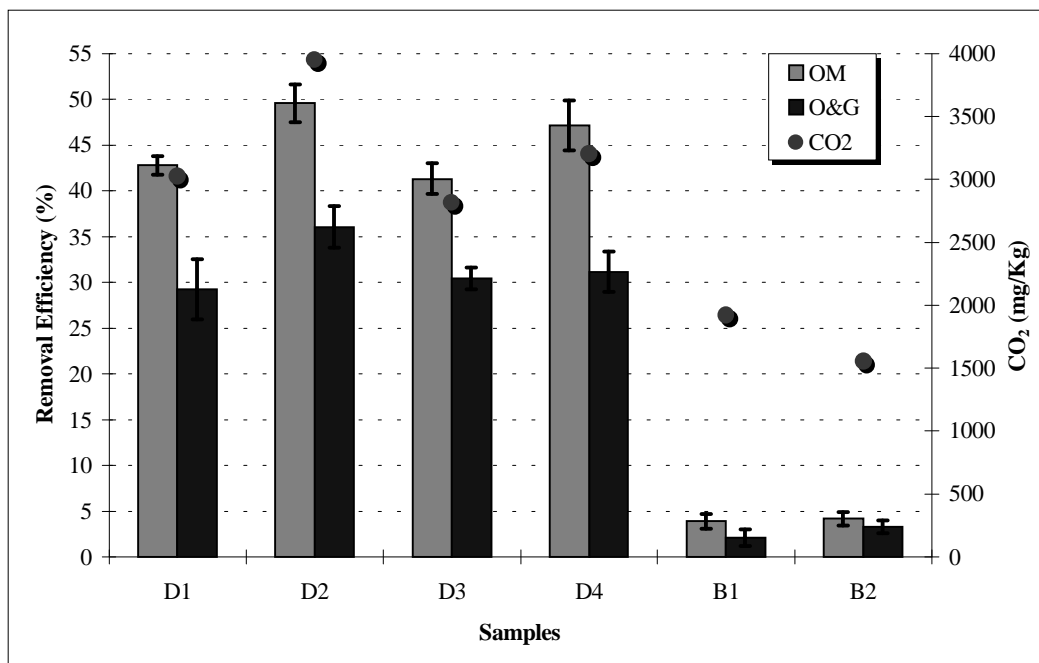


Figure 3 - Relationship between CO₂ Production and Organic Matter and OandG Removal Efficiency after 45 Days-Experiment (■ - MO, ■ - OandG).

The relationship between the production of CO₂ and efficient removals of organic matter and OandG, after the 45 days-experiment is shown in Fig. 3.

Fig. 3 showed a relationship between CO₂ production and organic matter and OandG removals, as CO₂ production increased with crude oil degradation. Highest biodegradation was reached in **D2** bioreactor, which corresponded to a 50% OM and 35% OandG removals and 4,000 mg CO₂ production per kg of soil. The results obtained from the control bioreactors (**B1** and **B2**), which not were nutritionally supplemented, the low CO₂ production could be attributed to CO₂ existing in the porous media (Prevedello, 1996) and/or from the incoming air, since OM and OandG removals were below 5%. Bioreactor **D4** showed the second highest level of OM and OandG removals, about

47% and 30%, respectively and CO₂ production was 3,200 mg CO₂/kg.

CO₂ evolution has been used as indicator of bacterial respiration rate, a product of the bioremediation process (Kao et al., 2001). Increase in dissolved CO₂ in a groundwater plume indicated the existence of biodegradation. Namkoong et al. (2002) also verified that the degradation of TPH was related to microbial respiration and the results were expressed as CO₂ production.

In order to evaluate hydrocarbons biodegradation in soil, the increase of microbial population after 45-days assay was monitored (Fig. 4).

The TPH and OandG removals were indeed related to the increase of hydrocarbon-degrading bacteria (BHC) present in the soil. This explained the best OM, TPH and OandG removals and CO₂ production obtained from **D2**, in which the highest increase for BHC population was found.

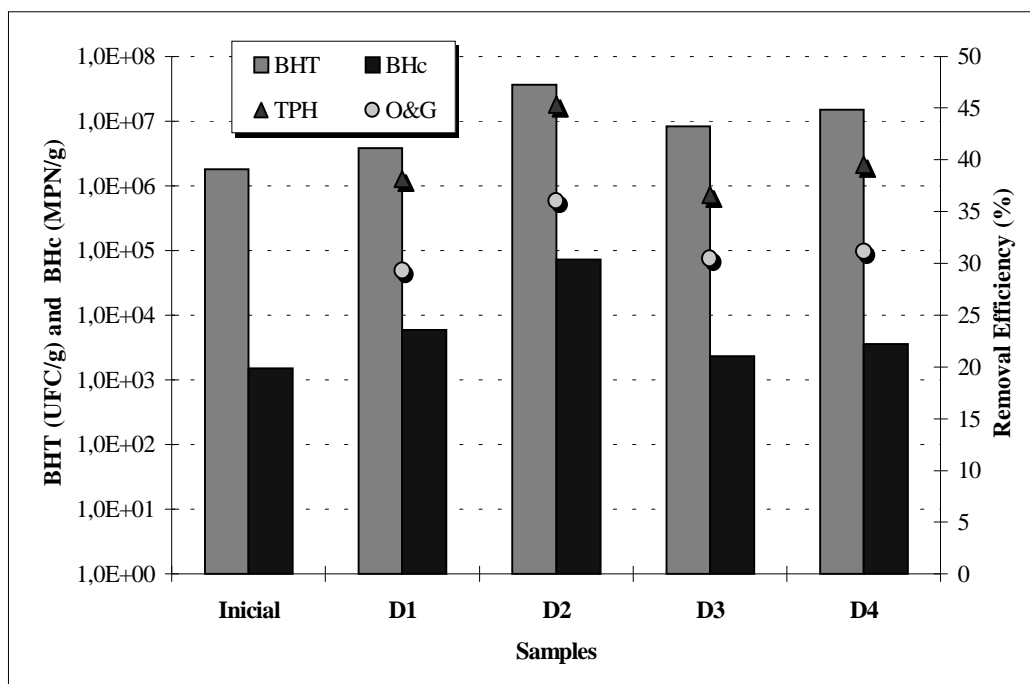


Figure 4 - Relationship between Increase of Microbial Population and Removal of TPH and OandG after 45-Days Experiment (■ - BHT, ■ - BHc).

Results of Figs. 3 and 4 showed that CO_2 production was also related to BHc increase, in the contaminated soil, since **D2** bioreactor showed the highest increase of BHc and CO_2 production per mass of soil. Phosphorus consumption was between 40-50%, while the nitrogen ranged from 25-35%. With regard to soil pH, before and after the 45-days experiment, it remained neutral.

Although crude oil removals in this work were shown low, the results were satisfactory, since the residual petroleum hydrocarbons in the soil investigated were considered recalcitrant, as the soil contamination took place in 2000. On the investigation of CO_2 production, it was evidenced that the bioreactors in which BHc increase reached the highest values were, as expected, the ones with the best removals of organic matter (50%), of OandG (37%) and TPH (45%), after the 45-days experiment.

ACKNOWLEDGEMENTS

The authors wish to thank Agência Nacional de Petróleo (ANP) for sponsoring this research and also CNPq/CTPETRO, FINEP/PADCT and FUJB

for their financial support to the Environmental Technology Laboratory at Escola de Química/UFRJ.

RESUMO

O objetivo do trabalho foi avaliar a biodegradação de petróleo em solo argiloso durante 45 dias de ensaios. Os ensaios de biodegradação foram conduzidos em biorreatores aeróbios de leito fixo, com 300 g de solo contaminado, à temperatura ambiente e com uma vazão de ar de 6 L/h. As deficiências nutricionais foram corrigidas com 2,5% (p/p) $(\text{NH}_4)_2\text{SO}_4$ e com 0,035% (p/p) KH_2PO_4 . O monitoramento foi realizado em função da produção de CO_2 , da remoção de matéria orgânica (OM), de óleos e graxas (OandG) e de hidrocarbonetos totais de petróleo (TPH), além bactérias heterotróficas totais (BHT) e hidrocarbonoclasticas (BHc), no início e após 45 dias. Nos biorreatores onde houve maior crescimento de bactérias hidrocarbonoclasticas e maior produção de CO_2 , obteve-se os melhores percentuais de remoções de MO (50%), OandG (37%) e TPH (45%).

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Received: September 29, 2004;
Revised: February 25, 2005;
Accepted: March 25, 2005.